using our derived equation for Y^s, and noting that assembly occurs abruptly when total active tubulin concentration equals affinity$_T^{-1}$ (H. Sternlicht et al., unpublished observations). This success lends further support to the validity of our Y^s equation.

CONCLUSION

Our analysis resulted in an expression for the composition of microtubule copolymers assembled in the presence of tubulin and CD. The affinity of the micrrotubule ends for both tubulin and CD correlates with the CD mole fraction in the microtubule phase, and the affinities decrease as CD_{total} increases. The molecular basis for these effects remains to be established.

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DNA CONDENSATION AND HOW IT RELATES TO PHASE EQUILIBRIUM IN SOLUTION

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High molecular weight DNA is a randomly coiled polymer usually found to be highly expanded in solution due to its low degree of flexibility. It has been shown, however, that DNA is able to undergo a sudden conformational transition into a highly compacted conformation. The collapse transition or condensation of DNA has been observed to be caused by a number of agents: polyamines (1), alcohol (2), acid (3), and polymer solutions such as polyethylene glycol (PEG) and polyacrylate (4). In a previous paper (5) we discussed the condensation of single molecules from the point of view of Flory's classical polymer solution theory (6). In this work we extend the discussion to take account of higher concentrations. Under these conditions the condensation can occur either as a unimolecular phenomenon or as an aggregation of many molecules leading to precipitation of the DNA. Condensation of single molecules and precipitation are thus viewed as two aspects of the effect of reduced solvent power and the accompanying lowering of the free energy of DNA-DNA and solvent-solvent contacts as compared with DNA-solvent contacts.

In the Flory theory the free energy of mixing of the solute (DNA) and the solvent is governed by a parameter χ. The χ value reflects the free energy of interaction of a solvent-segment contact, ignoring the molecular details of such contacts. In the free energy expressions the quantity that appears is $\chi - 1/2$, the $1/2$ arising from the usual expression for the entropy of mixing (Raoult's law). When $\chi < 1/2$ the free energy of mixing is negative

(except for some small terms that are not important with solutes of high molecular weight), and when $\chi > 1/2$ the free energy is positive and two phases tend to separate.

We have used Flory's equations, with the addition of a term for the free energy of the collapsed single molecules, to compute a phase diagram of the state of the solution as a function of χ and of concentration. The results for several DNA molecular weights are shown in Fig. 1. There are three possible states of the DNA. At low values of χ the interaction with the solvent favors mixing and the DNA molecule is in a randomly coiled state at all concentrations. As the value of χ increases, the attraction between the DNA segments leads to phase separation, either as dispersed but collapsed single molecules at low concentrations or as precipitated aggregates at high concentrations. There is a two-phase region at intermediate concentrations and high χ values. The values of DNA concentration and χ at which phase separation occurs are a function of DNA molecular weight. For higher molecular weight, separation occurs at smaller χ and lower concentrations.

The parameter χ can be thought of as analogous to temperature, though it is actually influenced by solvent composition as well. For a solvent with several components, for example, PEG and Na^+, the χ value reflects the average solvent environment. This allows a general treatment of the phase separation of DNA in any poor solvent, either polyamines, other polymers, or alcohol. In all cases, the phase transition is the result of a preference for like contacts over unlike contacts. There is a large tendency for DNA to self-associate, leading to an effectively repulsive force between DNA and solvent molecules.

A question is: can the collapsed form be experimentally studied under conditions in which it is a thermodynamically stable configuration and not a metastable one with respect to the aggregated state? Although the calculated phase diagram has not been compared yet with

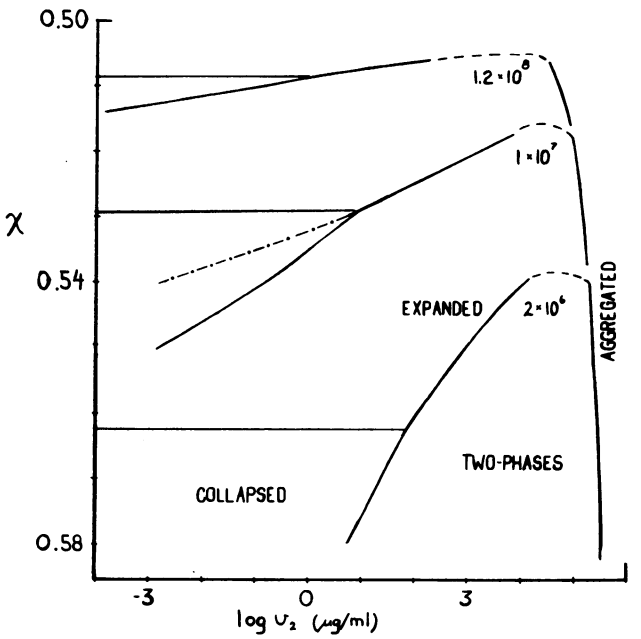

Figure 1 Phase diagram of DNA solutions. Coexistence curves are plotted for values of χ, the interaction parameter, as a function of the logarithm of the DNA concentration for three molecular weights (numbers on curves). The labeled regions refer to the curve for $M = 2 \times 10^6$ and indicate the conformational state of the DNA.

experiment, our calculations suggest that in a very dilute DNA solution, <5 μg/ml for a molecular weight of 1×10^7, the collapsed form can be a stable configuration.

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LAC REPRESSOR

A Genetic and Nuclear Magnetic Resonance Study of Structure and Function

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The prototype gene control system, the *lac* operon of *E. coli* (1), has recently also become the best chemically characterized system to date (2). The complete primary sequence of both the gene (3) and the protein (4) responsible for the regulation of this operon, the repressor, is known, along with the DNA sequence of its site of action (5), the operator. The *lac* repressor is a tetrametic protein with four identical subunits of 360 amino acids each, giving a total molecular weight of 154,000. The *lac* operator sequence is about 25–30 base pairs long. With the wealth of information about the primary structure the next question is one of geometry. This leads to the application of either x-ray diffraction or nuclear magnetic resonance (NMR) methods, since these are the only approaches that yield information about the geometry and environment of specific groups and atoms in these molecules. Since we are interested in the interaction of repressor with a variety of small molecular weight inducers and anti-inducers, as well as the operator sequence in aqueous solution, we chose the NMR approach. As of this writing, no useful crystals of the *lac* repressor or the repressor and any of its ligands have been reported. Because of our extensive genetic work with this system, we have a unique advantage in taking this approach as well.

Using a comprehensive mutagenesis and mapping scheme, we have isolated 90 nonsense mutations in the gene coding for the repressor protein (2, 6). It is possible to examine the properties of each of these nonsense mutations in all of the known nonsense suppressor backgrounds. We have looked at the effect of over 400 individual single amino acid alterations on repressor function (6). This ability to vary specific amino acids in specific locations in the protein allows a direct method for the assignments of features in the spectrum. In the case under consideration here, we have used the added expedient of looking initially